

CLAIMS

What is claimed is:

5 1. An *in vitro* system for use in identifying a modulator of neural differentiation, comprising:

- (a) a collection of embryonic stem cells;
- (b) a rostralizing or caudalizing embryonic signalling factor; and
- (c) optionally, a dorsalizing or ventralizing embryonic signalling factor.

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2. The system of claim 1, wherein the embryonic stem cells are murine embryonic stem cells.

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3. The system of claim 1, wherein the embryonic stem cells are human embryonic stem cells.

4. The system of claim 1, wherein the rostralizing embryonic signalling factor is a retinoid or Wnt.

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5. The system of claim 1, wherein the caudalizing embryonic signalling factor is a retinoid or Wnt.

6. The system of claim 1, wherein the dorsalizing embryonic signalling factor is a BMP or Wnt.

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7. The system of claim 1, wherein the ventralizing embryonic signalling factor is an activator of a hedgehog signalling pathway.

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8. The system of claim 7, wherein the activator of a hedgehog signalling pathway is a hedgehog protein or an agonist of hedgehog signalling.

9. The system of claim 7, wherein the activator of a hedgehog signalling pathway is DHh, IHh, SHh, or Hh-Ag1.3.

10. The system of claim 1, further comprising at least one neurotrophic factor.
11. The system of claim 1, further comprising at least one conditioned medium.
- 5 12. The system of claim 11, wherein the at least one conditioned medium is Wnt3a conditioned medium and/or mFrz8CRD conditioned medium.
13. A modulator identified by the system of claim 1.
- 10 14. A method for identifying a modulator of neural differentiation, comprising the steps of:
 - (a) contacting the *in vitro* system of claim 1 with a candidate modulator; and
 - (b) determining if the candidate modulator has an effect on neural differentiation of the embryonic stem cells.
- 15 15. A modulator identified by the method of claim 14.
16. An *in vitro* system for use in identifying a modulator of neural differentiation, comprising a collection of embryonic stem cells that has been contacted with:
 - (a) a rostralizing or caudalizing embryonic signalling factor, to produce a collection of neural progenitor cells; and
 - (b) optionally, a dorsalizing or ventralizing embryonic signalling factor.
- 20 17. The system of claim 16, wherein the embryonic stem cells are murine embryonic stem cells.
- 25 18. The system of claim 16, wherein the embryonic stem cells are human embryonic stem cells.
- 30 19. The system of claim 16, wherein the neural progenitor cells are selected from the group consisting of progenitor cells of DRG neurons, progenitor cells of motor neurons, progenitor cells of peripheral neurons, progenitor cells of spinal cord neurons, and progenitor cells of ventral interneurons.

20. The system of claim 16, wherein the neural progenitor cells differentiate into repositioned neural progenitor cells or fully-differentiated neural cells.

21. The system of claim 20, wherein the fully-differentiated neural cells are motor
5 neurons.

22. The system of claim 21, wherein the motor neurons are post-mitotic.

23. The system of claim 21, wherein the motor neurons are spinal motor neurons.

10 24. The system of claim 16, further comprising at least one conditioned medium.

25. The system of claim 24, wherein the at least one conditioned medium is Wnt3a conditioned medium and/or mFrz8CRD conditioned medium.

15 26. The system of claim 16, further comprising at least one neurotrophic factor.

27. A modulator identified by the system of claim 16.

20 28. A method for identifying a modulator of neural differentiation, comprising the steps of:
(a) contacting the *in vitro* system of claim 16 with a candidate modulator; and
(b) determining if the candidate modulator has an effect on neural differentiation of the neural progenitor cells.

25 29. A modulator identified by the method of claim 28.

30. A method for identifying a modulator of neural differentiation, comprising the steps of:

30 (a) obtaining or generating a collection of embryonic stem cells;
(b) contacting the embryonic stem cells simultaneously with a rostralizing or caudalizing embryonic signalling factor and a dorsalizing or ventralizing embryonic signalling factor, in the presence of a candidate modulator; and

(c) determining if the candidate modulator modulates neural differentiation of the embryonic stem cells.

31. The method of claim 30, wherein the embryonic stem cells are murine
5 embryonic stem cells.

32. The method of claim 30, wherein the embryonic stem cells are human
embryonic stem cells.

10 33. The method of claim 30, wherein the determination in step (c) is made by
comparing neural differentiation of the embryonic stem cells in step (b) with neural
differentiation of a second collection of embryonic stem cells that have been contacted with a
rostralizing or caudalizing embryonic signalling factor, and a dorsalizing or ventralizing
embryonic signalling factor, in the absence of the candidate modulator.

15 34. The method of claim 30, further comprising the step of contacting the
embryonic stem cells in step (b) with at least one neurotrophic factor.

20 35. The method of claim 30, further comprising the step of contacting the
embryonic stem cells in step (b) with at least one conditioned medium.

36. The method of claim 30, wherein the embryonic stem cells in step (b) are
contacted with the rostralizing or caudalizing embryonic signalling factor prior to contact
with the dorsalizing or ventralizing embryonic signalling factor.

25 37. A modulator identified by the method of claim 30.

38. A method for identifying a modulator of neural differentiation, comprising the
steps of:

30 (a) obtaining or generating a first collection of embryonic stem cells and a second
collection of embryonic stem cells;
(b) contacting the first collection of embryonic stem cells with a rostralizing or
caudalizing embryonic signalling factor and a dorsalizing or ventralizing embryonic
signalling factor, in the presence of a candidate modulator;

(c) contacting the second collection of embryonic stem cells with a rostralizing or caudalizing embryonic signalling factor and a dorsalizing or ventralizing embryonic signalling factor, in the absence of the candidate modulator; and

5 (d) determining if the candidate modulator in step (b) modulates neural differentiation of embryonic stem cells by comparing neural differentiation of the embryonic stem cells in step (b) with neural differentiation of the embryonic stem cells in step (c).

39. A modulator identified by the method of claim 38.

10 40. A method for identifying a modulator of neural differentiation, comprising the steps of:

(a) obtaining or generating a collection of embryonic stem cells;

(b) contacting the collection of embryonic stem cells with an amount of a rostralizing or caudalizing embryonic signalling factor effective to produce neural progenitor 15 cells;

(c) contacting the neural progenitor cells with a dorsalizing or ventralizing embryonic signalling factor, in the presence of a candidate modulator; and

(d) determining if the candidate modulator modulates neural differentiation of the embryonic stem cells.

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41. The method of claim 40, wherein the embryonic stem cells differentiate into fully-differentiated neural cells.

25 42. The method of claim 41, wherein the fully-differentiated neural cells are selected from the group consisting of astrocytes, neurons, oligodendrocytes, and Schwann cells.

43. A method for identifying a modulator of an FGF signalling pathway, comprising the steps of:

30 (a) obtaining or generating a first collection of cells selected from the group consisting of embryonic stem cells, embryoid bodies, and neural progenitor cells;

(b) obtaining or generating a second collection of cells selected from the same group as the cells selected in step (a);

- (c) contacting the first collection of cells with a candidate modulator, in the presence of an activator of an FGF signalling pathway;
- (d) contacting the second collection of cells with an activator of an FGF signalling pathway; and
- 5 (e) determining if the candidate modulator in step (b) modulates neural differentiation by comparing neural differentiation of the cells in step (b) with neural differentiation of the cells in step (c), wherein modulation of neural differentiation is indicative of modulation of an FGF signalling pathway.

10 44. The method of claim 43, wherein the embryonic stem cells are selected from the group consisting of murine embryonic stem cells and human embryonic stem cells.

45. The method of claim 43, wherein the neural progenitor cells in step (a) and step (b) are generated from embryonic stem cells.

15 46. The method of claim 43, wherein the cells in step (a) differentiate into repositioned neural progenitor cells or fully-differentiated neural cells.

20 47. The method of claim 46, wherein the fully-differentiated neural cells are motor neurons.

48. The method of claim 43, wherein the candidate modulator enhances neural differentiation.

25 49. The method of claim 43, wherein the activator of an FGF signalling pathway is selected from the group consisting of activin, AREG (amphiregulin), BDGF, BEK, BMP, brachyury, BSN-CM, BTL, chordin, c-met, CNTF, Crk, CSF1 (M-CSF), CSF2 (GM-CSF), CSF3 (G-CSF), CTGF, CXCL1 (MGSA), DAG, DTR (HB-EGF), E2A-Pbx1, ECGF1 (PD-ECGF), EGF, ELK-1, EPO, ERK1, ERK2, an FGF protein, an FGF receptor, FIGF (VEGFD), FRS2, GDNF, GH1, GH2, Grb2, Grb2-Sos, GTPase, heparin, HGF, HLGAG, HSPG, HTL, IGF1, IGF2, an interleukin, INS, IP3, KITLG (SCF), KLK2 (NGFA), LIF, LOC145957 (neuregulin 4), LTA (TNF beta), MAPK, MDK (midkine), MEK, MSX, NDK, NGFB, NRG1, NRG2, NRG3, NRP1 (neuropilin 1), NRP2, NT6G, NTF3, NTF5, PDGFA, PDGFB, PDGFC, PGF (PLGF), PI3 kinase, PIP2, PKC, PLC, PTN, RACK1, Raf kinase,

Ras, RTK, SCDGF-B (PDGFD), Shc, SHP1, SHP2, SNT-1, Sos, Spry, Src, TGFA, TGFB1, TGFB2, TGFB3, TNF, VEGF, VEGFB, VEGFC, WT1, Xbra, XER81, XFLRT3, Xpo, and any analogue or homologue thereof.

5 50. A method for identifying a modulator of neural differentiation, comprising the steps of:

- (a) obtaining or generating a first collection of cells selected from the group consisting of embryonic stem cells, embryoid bodies, and neural progenitor cells;
- (b) obtaining or generating a second collection of cells selected from the same group as the cells selected in step (a);
- (c) contacting the first collection of cells with a candidate modulator, in the presence of an activator of an FGF signalling pathway;
- (d) contacting the second collection of cells with an activator of an FGF signalling pathway; and
- 15 (e) determining if the candidate modulator in step (b) modulates neural differentiation by comparing neural differentiation of the cells in step (b) with neural differentiation of the cells in step (c).

20 51. A method for identifying a modulator of FGF-dependent neural differentiation, comprising the steps of:

- (a) obtaining or generating a collection of cells selected from the group consisting of embryonic stem cells, embryoid bodies, and neural progenitor cells;
- (b) contacting the collection of cells with a candidate modulator; and
- (c) determining if the candidate modulator modulates FGF-dependent neural differentiation of the cells in the collection.

25 52. The method of claim 51, wherein the collection of cells in step (b) is further contacted with the candidate modulator in the presence of an activator of an FGF signalling pathway.

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53. A method for identifying a modulator of a retinoid signalling pathway, comprising the steps of:

- (a) obtaining or generating a first collection of cells selected from the group consisting of embryonic stem cells, embryoid bodies, and neural progenitor cells;

(b) obtaining or generating a second collection of cells selected from the same group as the cells selected in step (a);

(c) contacting the first collection of cells with a candidate modulator, in the presence of an activator of a retinoid signalling pathway;

5 (d) contacting the second collection of cells with an activator of a retinoid signalling pathway; and

(e) determining if the candidate modulator in step (b) modulates neural differentiation by comparing neural differentiation of the cells in step (b) with neural differentiation of the cells in step (c), wherein modulation of neural differentiation is indicative of modulation of a retinoid signalling pathway.

10 54. The method of claim 53, wherein the embryonic stem cells are selected from the group consisting of murine embryonic stem cells and human embryonic stem cells.

15 55. The method of claim 53, wherein the cells in step (a) and step (b) are embryoid bodies, and wherein the cells in step (a) differentiate into neural progenitor cells.

56. The method of claim 55, wherein the neural progenitor cells further differentiate into motor neurons.

20 57. The method of claim 53, wherein the cells in step (a) and step (b) are neural progenitor cells of a midbrain character, and wherein the cells in step (a) differentiate into repositioned neural progenitor cells of a caudal character or repositioned neural progenitor cells of spinal interneurons.

25 58. The method of claim 57, wherein the repositioned neural progenitor cells of a caudal character or the repositioned neural progenitor cells of spinal interneurons further differentiate into motor neurons.

30 59. The method of claim 53, wherein the embryoid bodies in step (a) and step (b) are generated from embryonic stem cells.

60. The method of claim 53, wherein the cells in step (a) differentiate into repositioned neural progenitor cells or fully-differentiated neural cells.

61. The method of claim 60, wherein the fully-differentiated neural cells are motor neurons.

62. The method of claim 53, wherein the candidate modulator enhances neural
5 differentiation.

63. The method of claim 53, wherein the activator of a retinoid signalling pathway
is selected from the group consisting of Cbfa1, CDX1, CTSD (cathepsin D), Cyp26, EN1
(engrailed homolog 1), FLJ12541 (Stra6), HOX1, HOXA1, HOXB1, phorbol-12-myristate-
10 13-acetate-activated protein kinase C, a retinoid, RAR, RBP1 (CRBPI), RBP2 (CRABPI),
RXR, Sox9, and any analogue or homologue thereof.

64. A method for identifying a modulator of neural differentiation, comprising the
steps of:

15 (a) obtaining or generating a first collection of cells selected from the group
consisting of embryonic stem cells, embryoid bodies, and neural progenitor cells;
(b) obtaining or generating a second collection of cells selected from the same
group as the cells selected in step (a);
20 (c) contacting the first collection of cells with a candidate modulator, in the
presence of an activator of a retinoid signalling pathway;
(d) contacting the second collection of cells with an activator of a retinoid
signalling pathway; and
25 (e) determining if the candidate modulator in step (b) modulates neural
differentiation by comparing neural differentiation of the cells in step (b) with neural
differentiation of the cells in step (c).

65. A method for identifying a modulator of retinoid-dependent neural
differentiation, comprising the steps of:

30 (a) obtaining or generating a collection of cells selected from the group consisting
of embryonic stem cells, embryoid bodies, and neural progenitor cells;
(b) contacting the collection of cells with a candidate modulator; and
(c) determining if the candidate modulator modulates retinoid-dependent neural
differentiation of the cells in the collection.

66. The method of claim 61, wherein the collection of cells in step (b) is further contacted with the candidate modulator in the presence of an activator of a retinoid signalling pathway.

5 67. A modulator identified by any of the methods of claims 43, 50, 51, 53, 64, and
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